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Expression and Excretion of Human Fibroblast β_1 Interferon in Monkey Cells After Transfection with a Recombinant SV40 Plasmid Vector

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Summary: We have constructed a eukaryotic expression vector designed to express a gene under late SV40 transcriptional control. From this chimeric plasmic-SV40 vector, virtually all the sequences which code for the major capsid protein VP1 have been deleted and instead, the human fibroblast interferon β_1 cDNA gene has been inserted. After transfection of monkey cells with this recombinant, substantial quantities of human β_1 interferon (up to 2×10^{-4} IU/ml) were excreted in the culture medium. Transfection of nonpermissive mouse L cells or rat cells yielded virtually undetectable quantities of human β_1 interferon (5×10^3 to 10^4 times less than that in monkey cells). The recombinant SV40 vector may serve as a model vehicle for the efficient expression of other eukaryotic genes and might also be used as a direct screening vector for cloning of eukaryotic or prokaryotic cDNA genes. **Key Words:** Screening-vector—cDNA cloning—pSV529—Interferon.

SV40 recombinants are particularly attractive as vehicles for the expression of heterologous genes for a number of reasons (1–7). The virus genome consists of a single, small circular DNA molecule, and the entire nucleotide sequence of 5243 base pairs (bp) has been determined (8,9). The viral DNA is readily obtainable in large quantities (10), and the genomic regions responsible for the various viral functions have been accurately located on the detailed physical map (11). The viral genome can multiply to a high copy number in permissive cells or be maintained as an integral part of the cellular genome in transformed cells. Furthermore, SV40-plasmid chimeric vectors can be propagated in *Escherichia coli,* and if DNA transfections only are carried out, there is no constraint on the size of the cloned DNA. This is in contrast to the restrictions on the size of the recombinant genomes propagated as SV40 virions (with helper virus) where the DNA fragment inserted in the late region must be smaller than 2500 bp (12). Moreover, recent experiments (13,14) with a globin gene have demonstrated the importance of SV40 enhancer sequences located on the 72 bp tandem repeat on the late SV40 region. These sequences enhance the expression of linked foreign inserted genes.

We report here on the construction of a eukaryotic SV40 plasmid which may serve as a

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vehicle for the efficient expression of a foreign gene under the control of SV40 late transcription. In this chimeric plasmid-SV40 vector, pSV529, virtually the whole of the coding sequence for the major capsid protein VP1 [0.945 to 0.145 map units (15,16)] has been deleted and a foreign gene can be inserted in this position.

Since the SV40 DNA early region is partially duplicated in this vector (about 39% of the SV40 genome), homologous recombination occurs readily in the monkey cell, and this alleviates the need for elimination of the prokaryotic pBR322 DNA sequences by prior *in vitro* treatment with a restriction enzyme. As an example we introduced the human fibroblast interferon β_1 cDNA gene in the unique restriction site of pSV529 (17). The resulting recombinant can be propagated in *E. coli,* and after isolation of plasmid DNA and transfection of AP8 monkey cells, there is production and excretion of up to 2.10^{-4} IU/ml of human fibroblast β_1 interferon in the culture medium.

METHODS

Cell Culture and DNA Transfection

Kidney cells of the African Green Monkey (AP8), mouse L-tk$^-$ cells, and rat fibroblasts (F111) were maintained in Dulbecco's modified Eagle's minimal medium (DME) (GIBCO). The medium contained 10% newborn calf serum (GIBCO), 100 units of penicillin per ml, and 100 μg of streptomycin per ml. The cell cultures were transformed with plasmid DNA using either the calcium phosphate coprecipitation technique essentially as described (18) or using a modification of the DEAE-dextran method of McCutchan and Pagano (19,20). The following steps were carried out: the confluent cell monolayers were treated exhaustively with trypsin-EDTA for about 1 h and were then dispersed on 20 mm wells (24 wells per plate; Costar) in DME containing 10% newborn calf serum. After 8 h or 24 h, the cells were washed three times with serum-free DME, and the plasmid DNA (dissolved at about 1 to 10 μg/ml in 12 μl F11-HEPES buffer), was added to 120 μl DME containing 500 μg/ml DEAE-dextran (Pharmacia). This mixture was then applied to the cell monolayer for about 1–5 h or left overnight (in this case the concentration of DEAE-dextran was lowered to about 330 μg/ml) in a CO_2 incubator at 37°C. After the cells had been washed three times with DME, fresh medium (DME + 10% calf serum + 100 μg/ml penicillin and 100 μg/ml streptomycin) was added, and the cell cultures were incubated at 37°C in a CO_2 incubator for 2 to 3 days.

Plasmids and Enzymes

Plasmid DNA was prepared by a lysozyme-detergent lysis and purified by isodensity centrifugation in CsCl gradients in the presence of ethidium bromide essentially as described by Kahn et al. (21). Small-scale (20 ml) plasmid cultures were used to extract DNA by a modification of Birmboim and Dolly's (22) method. Preparation of intracellular SV40 DNA was according to Hirt's procedure (10).

Restriction enzymes were purchased from New England Biolabs or from Boehringer and were used as directed by the suppliers. Ligations were performed with T_4 ligase (Biolabs) or with a preparation made in our own laboratory. The ligation buffer contained 33 m*M* Tris, pH 7.8, 5 m*M* DTT, 10 m*M* MgCl, 0.1 m*M* EDTA, 10 μg/ml BSA, and 0.1 m*M* rATP for sticky ligation, and 0.5–1 m*M* rATP for blunt end ligation. The ligations were done at 15°C (for cohesive termini) or at 4°C (for blunt ends) using conditions as described previously (23).

Standard recombinant DNA techniques were employed with some modifications. The desired DNA fragments were fractionated by agarose gel electrophoresis after restriction digestion. The areas of low melting temperature agarose (BRL) which contained the DNA were cut out and the material was further purified by extraction and precipitation with isopropanol. Generation of a *Pvu*II partial digestion was carried out in a buffer containing 10 m*M* Tris, pH 7.6, 10 m*M* $MgCl_2$, 10 m*M* β-mercaptoethanol, 50 m*M* NaCl, and 33 μg/ml ethidium bromide

(with a DNA concentration of 100 μg/ml) and 2 units/ml *Pvu*II restriction enzyme. The digestion was allowed to proceed for 10 min at 37°C.

All work involving recombinant plasmids was done under conditions conforming to the Standard Guidelines of the Belgian Committee which are based on the NIH Guidelines for Recombinant DNA Research.

Assay for Human Interferon

Interferon assays were performed mainly as described (24,25). Human fibroblast (FS4) cells or human trisomic (T21) cells were seeded in Falcon's 96-well microtiter plates. Either encephalomyocarditis virus (EMC) or vesicular stomatitis virus (VSV) was used as a challenge virus for the production of cytopathic effect. Samples (which contained interferon and which had been frozen at −20°C until they were assayed) were applied after serial threefold dilution. EMC was added 20 h later. Plates were evaluated under a light microscope for inhibition of the virus-induced cytopathic effect using a human interferon β_1 preparation of known titer. Alternatively, the plates were stained with crystal violet. In order to characterize the interferon β_1 activity further, 20−50 μl aliquots of the samples (~ 10^3 units/ml) were incubated with 25 μl of goat antihuman fibroblast interferon for 60 min at 37°C, centrifuged at 1200 *g* for 5 min, and the supernatant was then assayed (25,26).

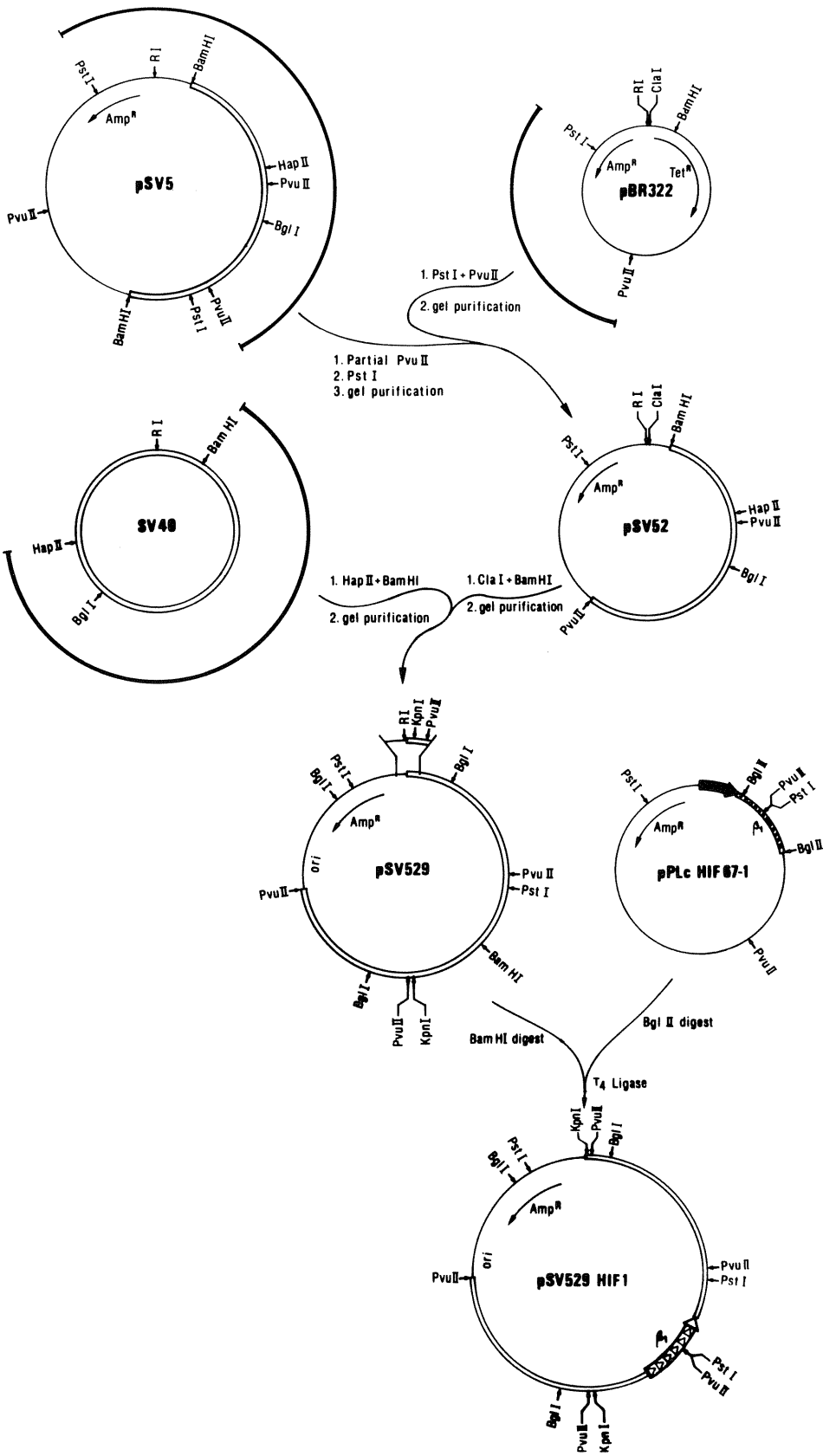
RESULTS

Construction of the SV40-Plasmid Expression Vector pSV529

The construction of a chimeric SV40 plasmid expression vector from which a substantial amount of the late region has been removed was similar to that of Mulligan et al. (2). The construction lacked most of the VP1 gene (between 0.945 and 0.145 map units), but it retained all the regions which are implicated in replication, initiation, and termination of transcription and in splicing and polyadenylation of 16S and 19S mRNA (27−31).

The construction of the expression vector pSV529 is outlined in Fig. 1. The chimeric plasmid pSV5 was kindly provided by Dr. C-J. Lai, and consists of the plasmid pBR322, into which SV40 DNA information has been inserted at the unique *Bam*HI site. This SV40 DNA sequence extends from position 0.945 (a *Hin*dIII site now converted to a *Bam*HI site) anticlockwise as far as the unique SV40 *Bam*HI site at position 0.145. Supercoiled plasmid pSV5 DNA was partially treated with *Pvu*II in the presence of ethidium bromide, and after phenolization and precipitation it was further digested with *Pst*I endonuclease. The 4330 bp long donor DNA fragment of pSV5 was gel purified and the same procedure was used for the 1542 bp long pBR322 *Pst-Pvu*II acceptor fragment (Fig. 1). The latter fragment was generated by a complete digestion of 5 μg of pBR322 DNA with *Pvu*II and *Pst*I endonucleases. The two purified fragments (donor and acceptor) were mixed in an equimolar ratio and ligated with T_4 ligase at 16°C. One-fifth of the ligation mixture was used to transform *E. coli* K12 strain HB1OI and more than 600 putative pBR322-SV40 recombinants were obtained. One of these clones, pSV52, which yielded the expected *Pvu*II fragments upon restriction analysis, was chosen for further study. It was additionally characterized by *Hin*dII and *Hin*dIII digestion and was tested for the presence of the unique *Bam*HI site (see Fig. 1).

The next step involved the digestion of pSV52 with *Cla*I and *Bam*HI endonucleases. The resulting 5520 bp long fragment was recovered from low melting point agarose. A *Bam*HI-*Hap*II fragment of 3096 base pairs spanning the early region of the SV40 genome (map units 0.145 to 0.725) was also gel purified. Although the *Cla*I (ATCGAT) and the *Hap*II (CCGG) endonuclease recognition sites differ, the cohesive ends (CG) generated by these endonucleases are identical. Accordingly, the 5520 bp long *Cla*I-*Bam*HI could be ligated to the gel-purified 3096 bp long *Hap*II *Bam* fragment (see legend to Fig. 1).


pSV5
Amp^R
Pst I
R I
BamHI
Hap II
Pvu II
Bgl I
pBR322
Amp^R
Tet^R
Cla I
1. Pst I + Pvu II
2. gel purification
1. Partial Pvu II
2. Pst I
3. gel purification
SV40
pSV52
1. Hap II + BamHI
2. gel purification
1. Cla I + BamHI
2. gel purification
pSV529
Kpn I
ori
pPLc HIF 67-1
BamHI digest
Bgl II digest
T4 Ligase
pSV529 HIF1

More than 500 carbenicillin-resistant clones were obtained after transformation of *E. coli* K12 (strain HB101). Six of eight recombinant clones analyzed on agarose gel were found to yield the desired fragments upon digestion with *Pvu*II, namely a 2341 bp, a 2263 bp, and two molar equivalents of a 2006 bp fragment. This is consistent with the expected repetition of a 2080 bp SV40 fragment from *Pvu*II (0.33) to *Hap*II (0.725) spanning the 5′-proximal half of the large T-antigen gene and the *ori* sequence of SV40.

One recombinant plasmid was chosen for further study and is named pSV529 (Fig. 2). Its main features are as follows. (a) The complete early region of the SV40 genome is present and codes for small-t and large-T antigens (32,33) as well as the region required for replication in monkey cells. Also present are the enhancer sequences (13) located toward the late region of *ori*. (b) The gene for the major structural protein VP1 has been deleted (the *Hin*dIII-*Bam*HI fragment from 0.945 to 0.145 map units). (c) A unique *Bam*HI-site is present on the chimeric plasmid into which foreign sequences can be inserted for expression under the control of the SV40 late promoter; this *Bam*-site is present 39 nucleotides after the late 16S mRNA acceptor splice site and 12 nucleotides before the (now removed) initiation codon of the VP1 gene. (d) Donor and acceptor splice sites for the major 16S late message are present (27,29). (e) A polyadenylation site from the SV40 late region is present [SV40 map units 0.17; (30)]. (f) Duplication of a 2080 bp segment of SV40 DNA (between map position 0.33 and 0.725) enables homologous recombination in monkey cells and generates an SV40 replicon from which the plasmid sequences have been deleted but which now contains the inserted gene instead of the viral structural gene VP1.

Construction of pSV529-HIF1

The human fibroblast β interferon cDNA gene (17) was then inserted into the unique *Bam*HI site of pSV529. The interferon cDNA segment of pPLcHFIF67-1 (25) was excised with *Bgl*II endonuclease (Fig. 2). This yielded a fragment of 667 bp which contained the whole of the coding portion of the gene including the codons for initiation of translation and termination (17). Even though the endonuclease recognition sites of *Bam*HI and *Bgl*II differ, the cohesive ends (GATC) generated are identical. This enabled the ligation of the 667 bp human interferon β cDNA fragment to the *Bam*HI-cleaved pSV529-vector (Fig. 1). After transformation of HB101 competent cells with 0.2 μg of the ligation mixture, more than 4000 carbenicillin-resistant clones were scored. Fifteen out of 16 recombinants analyzed contained the interferon-cDNA insert which was tested for by digestion with *Pvu*II and with *Pst*I. Both enzymes cut asymmetrically in the human β interferon cDNA fragment (17). One representative plasmid, pSV529-HIF1, containing the interferon gene in the correct orientation with respect to late transcription of SV40 was selected for further studies. Another plasmid, pSV529-HIF3, containing the same interferon gene but in the opposite orientation with respect to late transcription was also characterized and used as a control in further experiments.

FIG. 1. Construction of the expression vector pSV529 and the human fibroblast interferon containing plasmid pSV529-HIF1. Amp and Tet indicate ampicillin and tetracycline resistance genes. The numbers 1, 2, etc. indicate subsequent steps of the construction. The SV40 sequences are shown as the double-lined part of the circle, whereas human fibroblast β_1 interferon genes are shown as open blocks with arrows (indicating the sense orientation). Generation of pSV52 from pSV5 (a gift from Dr. C.-J. Lai) was by insertion of a gel-purified *Pvu*II (partial)-*Pst* fragment (4330 bp long indicated by the outer solid line). This fragment contained the truncated early region of SV40, the early and late promoter regions, a part of the late coding region till the *Hin*dIII site (0.945) now converted to *Bam*HI, and some pBR322 information. The gel-purified pBR322 acceptor fragment *Pst*I-*Pvu*II contained the *ori* and part of the penicillinase gene and is also indicated by an outer solid line. pSV529 was derived from pSV52 opened at the *Cla*I and *Bam*HI sites, followed by insertion of the gel-purified fragment *Hpa*II-*Bam*HI prepared from supercoiled SV40 DNA (outer solid line fragment). This resulted in a duplication of the SV40 early region from 0.725 to 0.33 in vector pSV529. The β_1 interferon harboring plasmid pSV529-HIF1 was generated by insertion of a *Bgl*II fragment from pPLcHIF67-1 (25), containing the whole coding part of the β_1 interferon gene into the *Bam*HI-digested pSV529 vector.

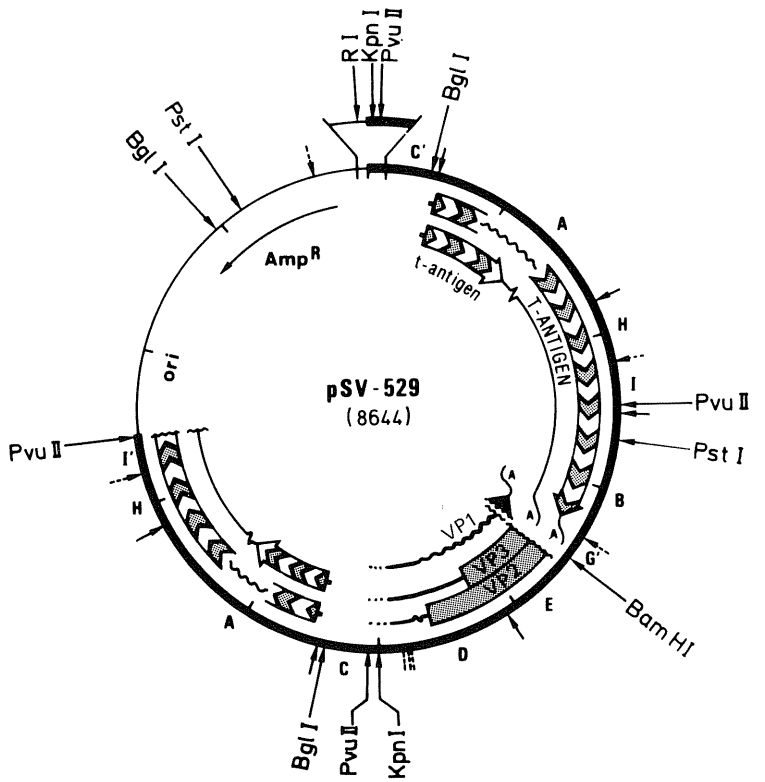


FIG. 2. A physical and genetic map of the expression vector pSV529. The thin segment part of the circle represents the 2300 bp long pBR322 DNA sequence. This extends from its unique *Cla*I restriction site (which is lost by joining to the *Hpa*II site of SV40) to the unique *Pvu*II. The sequence contains the ampicillin resistance gene and the origin of pBR322 replication. SV40 sequences are shown as a heavy line; a complete early region of the SV40 genome is present as well as the DNA replication region and the late region except that the gene for the major structural protein VP1 is almost completely deleted (0.945–0.145) but for the last 57 nucleotides at the 3′ translated end before the termination codon and about 140 nucleotides before the polyadenylation site. A unique *Bam*HI site was created at the deletion site (0.945) which enabled insertion of foreign genes under the control of the SV40 late transcription. The DNA replication and early region are partially duplicated (39% of the genome). The coding region for the early and late proteins are shown as shadowed arrows. The dotted 5′ ends and wavy poly-A 3′ ends indicate the span of the mRNAs. The jagged portions of each mRNA indicate the regions of the transcripts that are spliced out for forming the mature mRNAs. The lettering and small arrows on the outside refer to the *Hin*dII and III restriction fragments (8).

Formation of Human Fibroblast β_1 Interferon in Cultured Monkey Cells

In pSV529-HIF1 (Fig. 1) the human β_1 interferon cDNA fragment containing the AUG start codon and the UAA stop codon comes under the control of the SV40 late transcription system. The chimeric plasmid contains the SV40 donor and acceptor sequences implicated in splicing of the precursor to the major late 16S mRNA (27–29) and also the region where termination of late transcription and polyadenylation take place (30).

Transfection of AP8 cells was carried out by a modification of the DEAE-dextran method with either pSV529-HIF1 DNA or pSV529-HIF3 DNA, either alone or complemented with tsA58 virus at a multiplicity of five plaque forming units (PFU) per cell. The tsA58 helper virus is deficient in T-antigen synthesis at 40°C, but does produce the SV40 structural proteins VP1, VP2, and VP3.

Samples of 100 μl medium were taken at different times and were frozen at −20°C until their interferon titer could be determined on either FS4 or T21 human cells. It should be noted that the interferon gene also contained the signal sequence such that processing and excretion of the protein into the medium could be expected. The results shown in Fig. 3 do indeed demonstrate the production of human fibroblast β_1 interferon in cultured monkey cells after transfection with the pSV529-HIF1 recombinant. The interferon produced could be completely neutralized by antibody to human β_1 interferon (results not shown). Most of the interferon appeared in the medium between 48 and 72 h postinfection with pSV529-HIF1, and the titer reached was from 2×10^3 to 2×10^4 IU/ml depending on the quantity and nature of the DNA preparation used.

Figure 3 also shows that within the concentration range used, there is an almost linear relationship between the DNA input and the amount of interferon secreted into the medium of the monkey cells. After 72 h postinfection, production of interferon had virtually ceased (data not shown). The stability of β_1 interferon

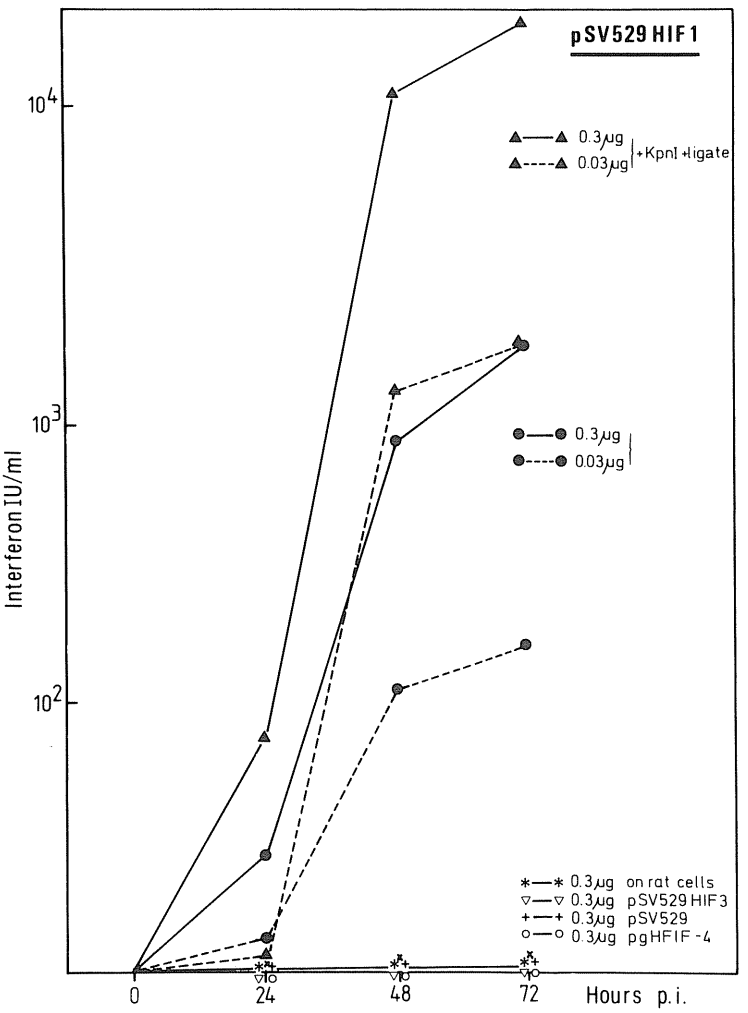


FIG. 3. Production of human fibroblast β_1 interferon in the culture medium of monkey cells transfected with pSV529-HIF1. About 10^5 AP8 monkey cells were transfected with different SV40 plasmid recombinants by a modification of the DEAE-dextran technique (see Methods). At the time indicated, 100 µl samples were withdrawn from the culture medium and frozen at −20°C until they were assayed for interferon. The detection limit of our interferon assay was 0.10 IU/ml or 0.30 IU/ml for human T21 cells and human FS4 cells, respectively. EMC was used as a challenge virus for induction of cytopathic effect. The error range is a factor of three. The β_1 interferon units are expressed as international units per ml (IU/ml). pSV529-HIF1 was treated with *Kpn*I and subsequently ligated in dilute solution; 0.3 µg DNA (▲——▲) or 0.03 µg DNA (▲– – –▲) was taken for transfection. pSV529-HIF1 without digestion and ligation: 0.3 µg DNA (●——●) or 0.03 µg DNA (●– – –●). Monkey AP8 cells were also transfected with 0.3 µg pSV529-HIF3 (+) which carries the *Bgl*II interferon insert in the opposite orientation with respect to the SV40 late promoter or with 0.3 µg pg-HFIF-4 (○) which carries the genomic β_1 interferon gene. pSV529-HIF1, 0.3 µg DNA, was also used to transfect rat cells (*) or human fibroblast FS4 cells (X).

is not much affected by prolonged incubation in the culture medium because even at 140 h postinfection, a β_1 interferon titer of 10^3 IU/ml was still detectable. Apparently the infection cannot spread after the first virus cycle even in the presence of helper virus. Dilute ligation of a *Kpn*I digest of pSV529-HIF1 which releases the SV40 replicon from the pBR322 sequences (see Fig. 1) results in a distinctly higher interferon titer. This difference is most pronounced at an intermediate input level (< 0.5 µg DNA per well). This finding may be explained by the presence of pBR322 sequences poisonous to replication of the plasmid in eukaryotic cells (34). Efficient expression was only obtained in cells permissive for SV40 (discussed below). The amount of SV40 late transcription may depend on an obligatory and direct coupling to replication and/or it can merely reflect the gene dosage.

Properties of pSV529

The formation of β_1 interferon is controlled by SV40 late transcription. pSV529-HIF1 retains all of the sequences involved in the biogenesis of SV40 late RNAs except for the region encoding the major capsid protein VP1 which is now replaced by the β_1 interferon structural gene. One could expect, therefore, that the expression of human β_1 interferon would depend on the orientation of the insert. Indeed, the results presented in Fig. 3 demonstrate that pSV529-HIF3 which contains the

same cDNA fragment, but in the opposite orientation, produced no detectable level of human fibroblast β_1 interferon (< 0.3 IU/ml) when assayed after 48 and 72 h. Transfection of SV40 nonpermissive rat or mouse cells with pSV529-HIF1 produced virtually no human β_1 interferon (assayed at 48 h postinfection). Addition of this same SV40-interferon recombinant to semi-permissive human FS4 cells resulted in the production of small, though reproducible, quantities of human interferon (1–5 IU/ml). Transfection of monkey cells with pgHFIF4, a recombinant derivative of pBR325 containing the genomic β_1 interferon DNA including 290 bp upstream the 5′ end of the mRNA (35,36), resulted in no detectable production of interferon (Fig. 3). When the latter plasmid was injected into the nuclei of *Xenopus laevis* oocytes, it produced intermediate levels of β_1 interferon compared to those produced by pSV529-HIF1 (H. Cheroutre and R. Contreras, unpublished results).

The human fibroblast interferon cDNA segment was also inserted into pSV529 by homopolymeric tailing with poly-dC at the 3′ site of the *Bam*HI endonuclease end of the vector and subsequent tailing of the β_1 interferon cDNA *Bgl*II fragment with poly-dG (17,37). The resulting plasmid pSV529-HIFG3 contained the same β_1 interferon insert in the same orientation but it was flanked by dG:dC tails and produced in AP8 cells a level of interferon in the medium which was four times less than that obtained in the absence of the dG:dC tails.

As a test of the use of this expression vector for direct selection of clones we grew up 50 colonies containing randomly cloned cDNA (derived from cellular mRNA) into pSV529 in the presence of one clone harboring pSV529-HIFG3. Transfection of monkey AP8 cells with this mixture of plasmid DNA resulted in a titer of $1-2 \times 10^3$ IU/ml of β_1 interferon. Clearly, this level of expression is far above the detection limit of 0.1 to 0.3 IU/ml of our interferon assay.

DISCUSSION

We constructed a eukaryotic expression vector pSV529 (Fig. 2) which may serve both as a vehicle for efficient expression of any eukaryotic gene in monkey cells and as a direct screening-expression vector for cDNAs of rare mRNAs. The vector consists of the SV40 late region, containing the mRNA initiation, donor, and acceptor splice sites and a polyadenylation site but not the coding region for the major structural protein VP1, the SV40 early region (which is duplicated over 39% of the SV40 genome), and also a pBR322 part with a prokaryotic *ori* and ampicillin-resistance gene. A foreign gene can be inserted into the unique *Bam*HI site (with or without poly dC:dG tailing,) and it replaces almost exactly the coding sequence for VP1. As an example we have introduced the human fibroblast β_1 interferon cDNA (17) into this chimeric SV40 plasmid vector. The resulting recombinant, after transfection of monkey cells, produced up to 2×10^4 IU/ml of human fibroblast β_1 interferon. This corresponds to about $1.8-2.5 \times 10^6$ molecules per cell, considering that at most only 20% of the monkey cells were transfected by the modified DEAE-dextran method (a specific activity of 5.10^8 IU/mg for human β_1 interferon was assumed) (38). Consideration of this high efficiency and the specificity of the host cell leads us to believe that the transcripts were initiated under SV40 late promoter control and were properly spliced. This conclusion is corroborated by experiments with pSV529-HIF3 which carries the β_1 interferon insert in the opposite orientation, by experiments in which we used pSV529-HIF1 in nonpermissive mouse cells and in semi-permissive human fibroblasts, and also by experiments with pgHFIF4 (see also Fig. 3). The fact that no enhancement was obtained by complementation with tsA58 virus (data not shown) leads us to suppose that the expression of human fibroblast β_1 interferon itself results in an antiviral protection for the monkey cells. Indeed the AGMK cells are protected against a subsequent SV40 infection by human α or β interferon. Also, the virus yield of the first virus cycle is drastically reduced (by up to 80%) if 10^2 units per ml of interferon is administered at 24 h postinfection and allowed to remain until harvest at 64 h postinfection. Moreover,

growing an SV40 virus stock on AP8 cells in the presence of human β_1 interferon (2.10^2 IU/ml) reduces the titer of the virus stock by several orders of magnitude. Apparently the antiviral activity of the human β_1 interferon can itself impose certain restraints upon the maximal level of expression which could theoretically be obtained with the pSV529 expression vector in monkey cells. We would expect that a cDNA construction which permits normal complementation with the tsA58 virus as helper for encapsidation and subsequent spreading of the infection would have a maximum expression level several orders of magnitude higher than that which we observed with human fibroblast β_1 interferon. Almost no human fibroblast β_1 interferon was synthesized in nonpermissive mouse L cells. But in semi-permissive human cells small but reproducible quantities of human fibroblast β_1 interferon were detectable.

The experiment in which we mixed fifty random pSV521 recombinants containing poly dC:dG cDNA-inserts with one pSV529-HIFG3 recombinant (see Results) clearly demonstrates that the signal strength of the expression of β_1 interferon was high enough ($1-2 \times 10^3$ IU/ml) above the detection limit of our assay. Hence it should be possible to use this vector to screen for a particular recombinant clone directly on the basis of expression of a biological, enzymatic, or immunological property. It should be noted that because of the partial duplication, homologous recombination in the monkey cell can readily be obtained. This avoids the need to eliminate the prokaryotic DNA sequences by pretreatment with a restriction enzyme; obviously such a procedure should be avoided when one is dealing with clones of unknown sequence. As an alternative to transfection of monkey cells, it is also possible to microinject the pSV529-derived recombinants directly into the nuclei of *X. laevis* oocytes and to assay the medium or the oocyte homogenate for biological activity.

It seems likely that expression systems of cloned genes in animal cells (e.g., by means of the vector pSV529), will provide a useful complement to prokaryotic systems. This is particularly so in those instances in which the gene product is modified and/or in which it is assembled into a complex biological structure.

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